In-vitro Disappearance of Carbohydrates, Phenolic Acids, and Lignin from Parenchyma and Sclerenchyma Cell Walls Isolated from Cocksfoot*

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ABSTRACT

Insight into the structure and digestion of the cell-wall matrix may be gained by studying the in-vitro disappearance (IVD) of constituents from cell types which are contained in forage fibre. The objective of this study was to determine the IVD of neutral sugars, uronic acids, esterified phenolic acids, and lignin from parenchyma and sclerenchyma walls isolated from the plant parts of cocksfoot (Dactylis glomerata L). Cell walls were incubated in a rumen fluid–buffer medium for 0, 12 or 96 h. The chemical composition, apparent digestion and IVD of constituents varied considerably within and between cell types. Parenchyma had higher arabinose to xylose and ferulic acid to p-coumaric acid ratios and lower lignin concentrations than sclerenchyma. The apparent digestion of parenchyma was 240% greater at 12 h and 20% greater at 96 h than that of sclerenchyma. The IVD of cell-wall constituents from parenchyma ranged from 12 to 80% at 12 h and 46 to 99% at 96 h. The IVD of constituents from sclerenchyma was lower than that from parenchyma, ranging from 5 to 50% at 12 h and 47 to 89% at 96 h. Across all cell types, IVD was usually greatest for ferulic acid and lowest for lignin. These findings indicate that the differential loss of constituents from fibre is due in part to differential loss of constituents from both fast and slowly digested cell types. Ferulic acid was the most uniformly removed constituent across cell types. This suggests that ferulic acid is

*associated with regions of the cell-wall matrix which change little between
cell types relative to structural carbohydrates and other lignins.*

INTRODUCTION

Insight into the digestion process of the cell-wall matrix may be gained by studying
the in-vitro disappearance (IVD) of its constituents. Most studies of the IVD of
cell-wall constituents have been made with heterogeneous preparations which
contain a mixture of cell types such as parenchyma and sclerenchyma. Such studies
indicate that all sugars except xylose and glucose are rapidly removed from cell
walls (Aman and Lindgren 1983; Gordon *et al* 1983; de Ruiter and Burns 1987a;
Wedig *et al* 1987). Core lignins (condensed polymers of cinnamyl alcohols) and
esterified *p*-coumaric acid resist hydrolysis by rumen–microbial enzymes (Chestnut
et al 1986; Jung 1989). However, another phenolic constituent, esterified ferulic
acid, is rapidly removed from cell walls and is positively correlated with digestibility
(Chestnut *et al* 1986; Buxton and Russell 1988).

Recent work indicates that differences in the relative IVD of cell-wall constituents
from heterogeneous cell-wall preparations are confounded by selective digestion
of cell types such as parenchyma while other cell types such as sclerenchyma
accumulate in the indigestible residue (Chesson *et al* 1986; Akin 1989). Parenchyma
and sclerenchyma walls have very different chemical compositions (Gordon *et al*
1985; Grabber *et al* 1991) and changes in the proportions of these cell types during
digestion can lead to misleading results when heterogeneous preparations are used
to study the relative IVD of cell-wall constituents. Analysis of isolated cell types
may provide a more definitive means of studying the chemistry and digestion of
forage fibre.

Reports of IVD of cell-wall constituents from isolated cell types have been
limited to neutral sugars in leaves of ryegrass (*Lolium* spp) and polysaccharides
in stems of forage kale (*Brassica oleracea* L) (Chesson *et al* 1986; Wilson *et al*
1989). The objective of this study was to determine the IVD of neutral sugars,
uronic acids, esterified phenolic acids, and lignin from parenchyma and
sclerenchyma walls isolated from the plant parts of cocksfoot.

EXPERIMENTAL

Plant material and cell-wall preparation

Herbage of cocksfoot (*Dactylis glomerata* L), at the early-heading growth stage,
was harvested on 30 May 1986 and on 28 May 1987 as described in Grabber *et
al* (1991). Parenchyma and sclerenchyma walls were isolated from homogenates
of leaf blades, leaf sheaths and stems by a wet-sieving technique (Grabber and

Jung 1991; Grabber *et al* 1991). Cell walls from four field replicates were combined each year to provide sufficient material for analyses. Parenchyma and sclerenchyma walls were milled in a vibratory-ball mill for 5 and 10 min, respectively, to a similar particle size ($< 150 \, \mu$m). Sufficient quantities of stem parenchyma from 1986 were not available for this study.

In-vitro disappearance of chemical constituents

The IVD of chemical constituents was determined by incubating duplicate cell-wall samples (0·175 g) in tared centrifuge tubes with 21 ml of rumen fluid/buffer inoculum which contained 0·5 g litre^{-1} of urea. Samples were hydrated for 45 min in 4·2 ml of phosphate buffer (Marten and Barnes 1980). A rumen fluid/buffer inoculum was then added which consisted of 4·2 ml of strained rumen fluid and 12·6 ml of phosphate buffer containing particle-associated microorganisms (Craig *et al* 1984). Samples were incubated for 0, 12 and 96 h. The 12 and 96 h time periods were selected to provide an estimate of the rate and extent, respectively, of IVD. After the appropriate time period, digestion was halted by adding 2·1 ml of ethanol and placing the tubes in iced water.

Cell-wall residues were collected by centrifuging tubes at $3000 \times g$ for 15 min. Residues were washed twice with water and the pellets from the final centrifugation were dried in an oven at 70°C for 3 days. A series of inoculum blanks were processed in a similar manner for each time period.

Neutral sugars, acetyl-bromide lignin, and esterified phenolic acids in cell-wall residues and inoculum residues were determined on duplicate samples by methods described in Grabber *et al* (1991). Uronic acids in acid hydrolysates from the neutral-sugar analyses were determined by the method of Blumenkrantz and Asboe-Hansen (1973). The composition of cell-wall residues were corrected for contamination from the inoculum based on chemical analyses of inoculum blanks.

Data from the IVD analyses were sorted by plant part and cell type. The year × chemical constituent interaction was used to test digestion differences between chemical constituents. Fisher's protected LSD was used to separate means when F ratios were significant at the 0·05 level of probability (SAS Institute 1985). The IVD of galactose from sclerenchyma was not reported because its concentration at 12 and 96 h could not be consistently quantified by packed-column gas chromatography.

RESULTS AND DISCUSSION

Composition and apparent digestion of cell walls

Analyses of cell walls accounted for 80 to 94% of dry matter (Table 1). The remaining cell-wall material would consist of proteins, ash, acetyl groups, and some cellulosic glucose which is not accounted for by neutral-sugar analyses (Gordon *et al* 1985). Parenchyma had higher concentrations or ratios of arabinose, galactose, uronic acid and ferulic acid to *p*-coumaric acid, and lower concentrations of xylose and lignin than sclerenchyma.

Parenchyma walls were rapidly and extensively digested in all plant parts (Table

TABLE 1

Chemical composition (g kg⁻¹ cell wall) at 0 h and apparent digestion (AD) of parenchyma (Par) and sclerenchyma (Scl) cell walls isolated from plant parts of cocksfoot, averaged over 2 years

Attribute[a]	Leaf blade		Leaf sheath		Stem	
	Par	Scl	Par	Scl	Par[b]	Scl
Arabinose	75 (0·1)[c]	28 (0·6)	75 (0·1)	26 (0·4)	38	14 (0·2)
Xylose	120 (2·7)	332 (4·2)	149 (3·6)	282 (1·0)	194	249 (3·3)
Galactose	26 (2·00)	3 (0·07)	24 (0·16)	3 (0·41)	7	1 (0·02)
Glucose	442 (11·6)	448 (2·39)	528 (13·4)	478 (2·4)	553	521 (3·9)
UA	86 (0·3)	43 (1·5)	111 (1·4)	53 (2·2)	71	44 (0·5)
PCA	1·6 (0·16)	4·5 (0·50)	2·3 (0·34)	6·8 (0·25)	7·8	6·8 (0·25)
FA	2·2 (0·03)	4·6 (0·19)	7·6 (0·17)	4·6 (0·11)	9·1	4·0 (0·24)
Lignin	50 (1·7)	83 (1·3)	42 (2·2)	90 (2·4)	62	90 (2·7)
12-h AD	489 (23)	226 (17)	524 (23)	189 (20)	364	153 (26)
96-h AD	799 (27)	705 (7)	841 (7)	643 (23)	731	642 (26)

[a] UA = uronic acid, PCA = esterified p-coumaric acid, FA = esterified ferulic acid.
[b] Values for 1987 only.
[c] Standard error of the mean (n = 2).

1); however, the rate of digestion was less than that reported by Chesson *et al* (1986) for leaf-mesophyll walls isolated from ryegrass. Sclerenchyma walls were digested much more slowly than parenchyma walls; this was also observed by Chesson *et al* (1986). However, the apparent digestion of sclerenchyma at 96 h (642–705 g kg^{-1}) was much greater than that observed in histological studies (Akin 1989). The digestion of sclerenchyma in our study was enhanced by separating it from adjacent tissues and by ball milling. After isolation, sclerenchyma walls were up to 10^5 times longer than parenchyma walls (Grabber J H, Jung G A, unpublished). Sclerenchyma walls were therefore ball milled for a longer period to make their surface area more comparable to that of parenchyma. Ball milling does, however, destroy the anatomical structure of sclerenchyma and may cause minor changes in polymer structure and solubility. The chemical composition and apparent digestion of cell types also differed between plant parts. Details of the chemical composition and digestion kinetics of these cell types were reported elsewhere (Grabber 1989; Grabber *et al* 1991).

In-vitro disappearance of constituents from cell walls

The IVD of constituents was greater from parenchyma walls than from sclerenchyma walls and greater for cell types from leaf blades and leaf sheaths than those from stems (Fig 1). The IVD of constituents also varied considerably within each cell type, particularly for stem parenchyma where the IVD of constituents ranged from 12 to 74% at 12 h and 46 to 95% at 96 h. In most cell types, the 12 h IVD of arabinose and uronic acid was greater ($P < 0.05$) than that of other carbohydrate constituents. After 96 h of digestion, the IVD of most carbohydrate constituents was similar in leaf blade and leaf sheath parenchyma. In contrast, the extent of xylose digestion was usually less ($P < 0.05$) that that of other carbohydrate constituents in stem parenchyma and in all sclerenchyma cell types.

Ferulic acid was consistently among the most rapidly and extensively removed constituents from cell walls (Fig 1). Our results and those of Buxton and Russell (1988) indicate that ferulic acid, unlike other lignins, does not structurally impede the digestion of cell walls. Ferulic acid also varied much less in its IVD across cell types than other cell-wall constituents. This suggests that ferulic acid is associated with regions of the cell-wall matrix which change little between cell types relative to other constituents.

Lignin and in many cases *p*-coumaric acid had the lowest ($P < 0.05$) IVD of cell-wall constituents (Fig 1). The extent of lignin IVD, as determined by the acetyl-bromide method, appeared high (46–69%) given that lignin is considered to be indigestible (Van Soest 1982). Apparent in-vivo lignin digestibilities of up to 43% have, however, been reported for permanganate lignin in cocksfoot (Chestnut *et al* 1986). A large proportion of lignin may be released from fibre during digestion (Conchie *et al* 1988). The release of lignins from cell walls in our study may have been enhanced by the small particle size of cell types. These lignins were probably not recovered during centrifugation when residues from digestion were collected. The IVD of lignin, *p*-coumaric acid and carbohydrate constituents varied considerably within and between cell types. These constituents are
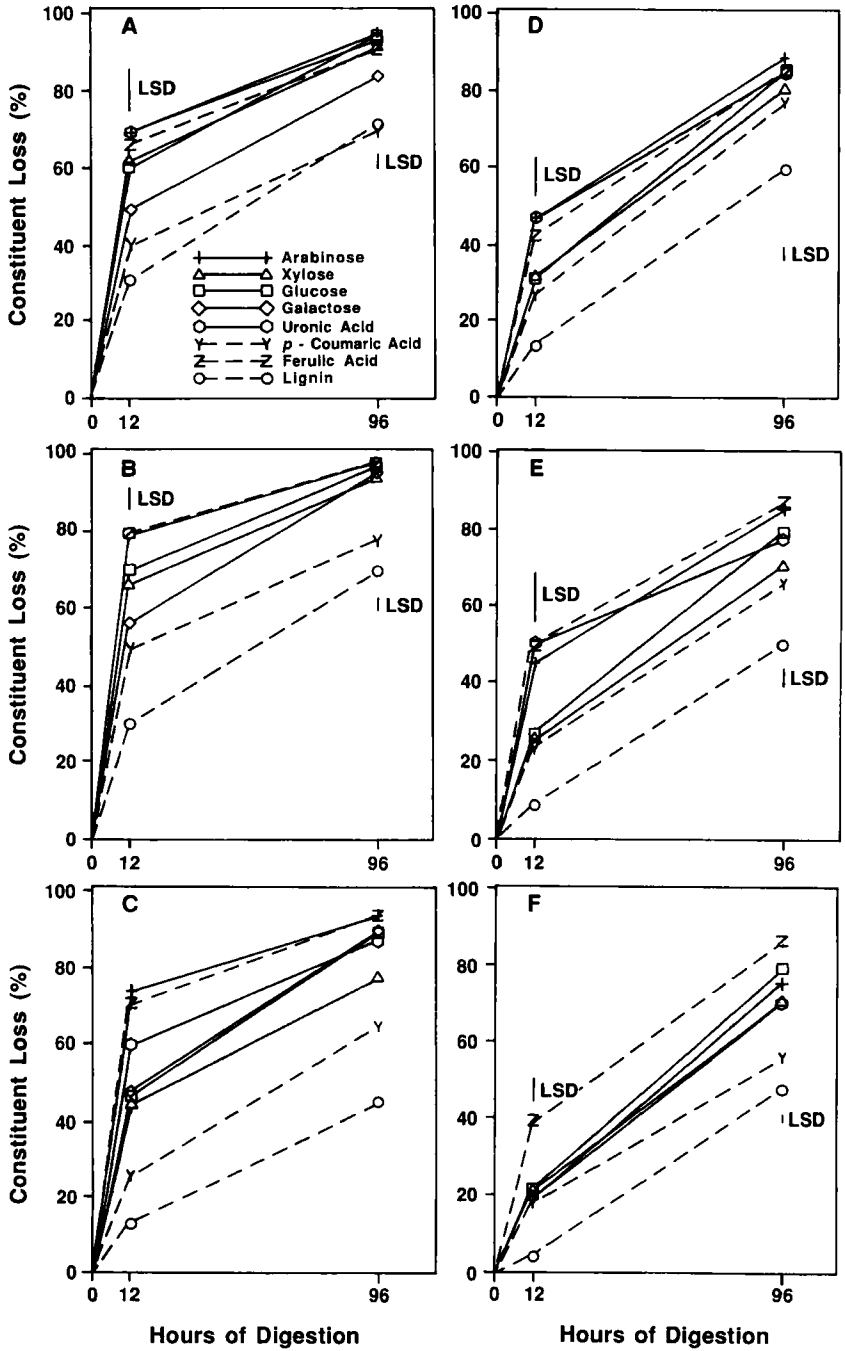
Fig 1. Loss of constituents from cocksfoot cell walls incubated in a rumen fluid–buffer medium for 12 and 96 h (duplicate determinations averaged over 2 years). A, Leaf blade parenchyma; B, leaf sheath parenchyma; C, stem parenchyma (1987 only); D, leaf blade sclerenchyma; E, leaf sheath sclerenchyma; F, stem sclerenchyma. LSD ($P < 0.05$).

apparently involved in various matrix interactions which influence their bioavailability (Das *et al* 1981; Chesson *et al* 1983; Mueller-Harvey *et al* 1986; Hatfield 1989; Jung 1989).

The concentration of constituents has been used to predict the digestion characteristics of heterogeneous fibre (Burritt *et al* 1985; de Ruiter and Burns 1987b). The variation in the concentration and IVD of constituents we observed suggests that chemical bonds, matrix interactions, or three-dimensional structures differ among and between cell types of cocksfoot. Our results suggest that the concentration of constituents in heterogeneous fibre preparations will not be highly related to the chemical composition or digestibility of all cell types contained in forage fibre.

The results of this study also indicate that changes in the proportions of constituents during fibre digestion are due in part to differential loss of constituents from both fast and slowly digested cell types. The differential IVD of constituents that we observed from sclerenchyma does not support the view that lignin affects all components of the cell wall equally (Chesson 1984; Chesson *et al* 1986).

These methods, while useful in examining the digestion characteristics of two diverse cell types contained in fibre, describe only a portion of a fibre fraction which contains many other cell types. Although isolated cell types are more chemically uniform than heterogeneous fibre preparations such as neutral-detergent fibre, the maturity of cell types and the composition of their cell walls varies between different levels of the plant canopy (Wilson 1976a,b; Akin *et al* 1977). In future studies, characterisation of fibre could be improved if cell types were isolated from specific levels of the plant canopy.

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